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Effects of Vagotomy and Bacterial Lipopolysaccharide on Food Intake and Expression of Cyclooxygenase-2 mRNA in Rat Brain Vessels

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Effects of bilateral subdiaphragmatic vagotomy on food intake and expression of cyclooxygenase-2 mRNA in cerebral vessels in rats intraperitoneally injected with bacterial lipopolysaccharide were studied using *in situ* hybridization technique. Low doses of lipopolysaccharide decreased food intake in sham-operated animals, but did not affect this parameter in vagotomized rats. Comparison of hybridization signals in brain slices showed that low doses of endotoxin did not affect expression of cyclooxygenase-2 mRNA in vessels of control and experimental animals. High doses of lipopolysaccharide reduced food intake in vagotomized and sham-operated rats and elevated cyclooxygenase-2 mRNA expression in vascular endothelial cells of the brain parenchyma and meninges. The data suggest that the vagus nerve activates central structures responsible for manifestation of anorexia after intraperitoneal injection of low doses of lipopolysaccharide. High doses of endotoxin activate the vagus-independent mechanism of cyclooxygenase-2 synthesis in the endothelium of cerebral vessels. It is assumed that prostaglandins synthesized by cyclooxygenase-2 diffuse into the brain parenchyma and cause anorexia by activating target nerve structures.

Key Words: *vagus nerve; vagotomy; cyclooxygenase; food intake*

Activation of the immune system after bacterial invasion or tissue damage causes a variety of body responses, including fever, changes in the secretion of pituitary and adrenal gland hormones, sleep-awareness disturbances, and anorexia [12]. These reactions controlled by the central nervous systems (CNS) are induced by cytokines secreted by antigen-stimulated immunocompetent cells. At the same time, it is known that large and hydrophilic cytokine molecules cannot cross the blood-brain barrier (BBB) and, therefore, produce only indirect effects on CNS [5,15].

Cytokines can stimulate the synthesis of short-living substances (*e.g.*, prostaglandins) in the vascular wall, which diffuse through the BBB into the brain parenchyma [12,15]. Animal experiments showed that the content of prostaglandins in the intercellular space increases after peripheral administration of bacterial endotoxin lipopolysaccharide (LPS), soluble component of bacterial walls from gram-negative bacteria [13]. Pharmacological blockade of prostaglandin synthesis suppresses activation of the hypothalamic-pituitary axis [10], sympathetic system, and serotonin- and norepinephrine-mediated transmission in the hippocampus [9] induced by LPS. These data suggest that production of prostaglandins in the brain is a key factor in CNS response to inflammatory agents.

Cyclooxygenase (COG) is an enzyme catalyzing conversion of arachidonic acid to prostaglandin H_2. There are 2 isoforms of this enzyme, COG-1 is a con-

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stitutive enzyme, whose level does not depend on immune stimulation. The expression of COG-2 is normally low, but increases after treatment with cytokines or LPS [4]. Recent studies indicate that COG-2 mRNA is induced in BBB cells after peripheral administration of LPS [2,3]. However, the data on the phenotype of COG-2-producing cells are ambiguous. Some authors reported that during inflammation prostaglandins are produced by cerebral vessel endotheliocytes, while others believe that these cells are of the microglial nature [6].

There is a great body of data on the important role of visceral afferents in mediating the effects of cytokines on the nervous system. It was shown that the vagus nerve transmits signals of abdominal inflammatory factors. Bilateral subdiaphragmatic vagotomy abolished hyperalgesia [16], somnolence, depression of communicative activity [1], and fever [14] induced by peripheral injection of LPS.

These data suggest that the interaction between cytokines and CNS is activated under various experimental conditions, including infections. To check this hypothesis, the role of the vagus nerve and prostaglandin synthesis in manifestations of central components of the acute phase of inflammation produced by various doses of LPS was studied. Anorexia served as the index of CNS reactivity to immune stimulation as the most typical sign of inflammatory process [8,11]. Here we analyzed the effects of vagotomy on anorexia induced by intraperitoneal injection of LPS in various doses. COG-2 mRNA expression in the brain after treatment with LPS and vagotomy was studied by *in situ* hybridization technique.

MATERIALS AND METHODS

Experiments were performed on 24 male Sprague-Dawley rats weighing 300-350 g. The rats were kept under standard light-dark cycle (daytime 6.00-18.00) at 20-22°C with *ad libitum* food and water supply. Four weeks before the experiment, 12 rats underwent vagotomy (experimental group) and 12 rats were sham-operated (control group). The animals were anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), and after longitudinal laparotomy the liver, stomach, and lower portion of the esophagus were removed and covered by sterile cloth moistened with physiological saline. Nerve trunks were separated from the esophageal serous membrane under the microscope, and the anterior and posterior branches of the vagus nerve were transected 10 mm below the diaphragm. Twenty-eight days after vagotomy, the effect of LPS on food intake were studied. Experiments were conducted in nighttime (18.00-20.00), when rodents demonstrated active feeding behavior. Vagotomized and sham-operated animals were intraperitoneally injected with low (5 μg/kg, 4 rats per group) and high (125 μg/g, 4 rats per group) doses of LPS (Sigma) in 1 ml 0.9% NaCl. Four vagotomized and 4 sham-operated rats received intraperitoneal injections of sterile physiological saline (1 ml). Dry food was weighted before and after the experiment. Four hours postinjection the rats were decapitated, the brain was rapidly removed and frozen for *in situ* hybridization assay. Hypothalamic slices (14 μ) were mounted on "Probe on" slides (Fisher Sci), dried, and incubated with a radiolabeled probe complementary to 1-34 nucleotide sequences of rat COG-2 mRNA (Scandinavian Gene Synthesis) at 42°C for 16 h. The probe was diluted with a solution containing 50% formamide, 0.015 M citrate buffer, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 1% N-lauroylsarcosine, 10% dextran, 500 mg/liter denatured salmon testes DNA, and 20 mM dithiothreitol. After hybridization, the slices were washed (1 h) with citrate buffer at 55°C, dried on air, and covered with an Ulford emulsion. After 3-week exposure, the slices were developed in a Kodak D19 solution, fixed in a Kodak 3000 solution, and stained with toluidine blue. The number of silver grains above labeled cells was calculated using a Nikon Microphot-FX microscope equipped with a dark-field condenser. The cell was considered to be labeled if the number of silver grains 5-fold surpassed that in the control. We calculated no less than 200 cells in brain slices from each rat. The results were analyzed by Student's *t* test.

RESULTS

Four hours after injection of LPS in the low dose, food intake in rats with intact vagal innervation sharply decreased compared to that in sham-operated animals administered 0.9% NaCl ($p<0.05$). Food intake in vagotomized rats remained unchanged after administration of LPS in the low dose. LPS in the high dose significantly decreased food intake in vagotomized and sham-operated rats ($p<0.01$, Fig. 1, *a*).

In brain slices of vagotomized and sham-operated rats injected with the low dose of LPS, we found only small amounts of autoradiographic labels corresponding to COG-2 mRNA localization in cerebral vessels. In animals receiving the high dose of LPS, expression of COG-2 mRNA ($p<0.001$, Fig. 1, *b*) in cells lining the luminal surface of cerebral and meningeal vessels considerably increased (Fig. 2).

These data show that vagotomy blocks anorexia caused by intraperitoneal administration of low, but not high doses of LPS. The distribution of autoradiographic labels above rat cerebral vessels indicates that high doses of LPS activate COG-2 synthesis in endotheliocytes. It can be assumed that prostaglandins pro-

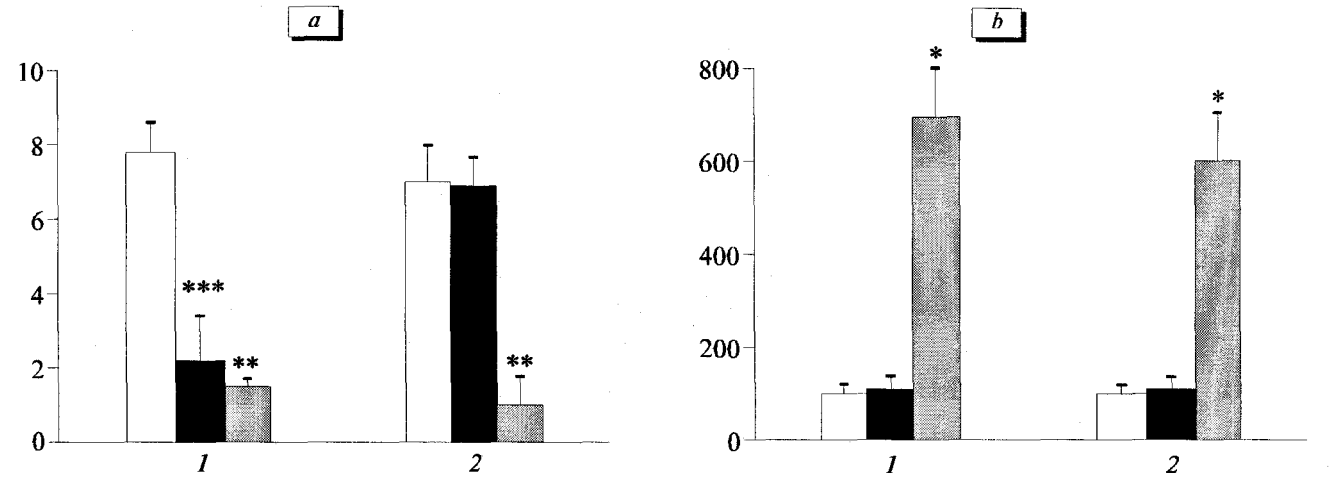

Fig. 1. Effects of low (5 μg/kg, dark bars) and high (125 μg/kg, shaded bars) doses of LPS on food intake (*a*) and expression of cyclooxygenase-2 mRNA (*b*) in cerebral vessels of rats with intact vagal innervation (*1*) and after bilateral subdiaphragmatic vagotomy (*2*). Ordinate: food consumption over 4 h postinjection (*a*) and mean number of silver grains above the cell (% of the control, *b*). *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control (0.9% NaCl, light bars).

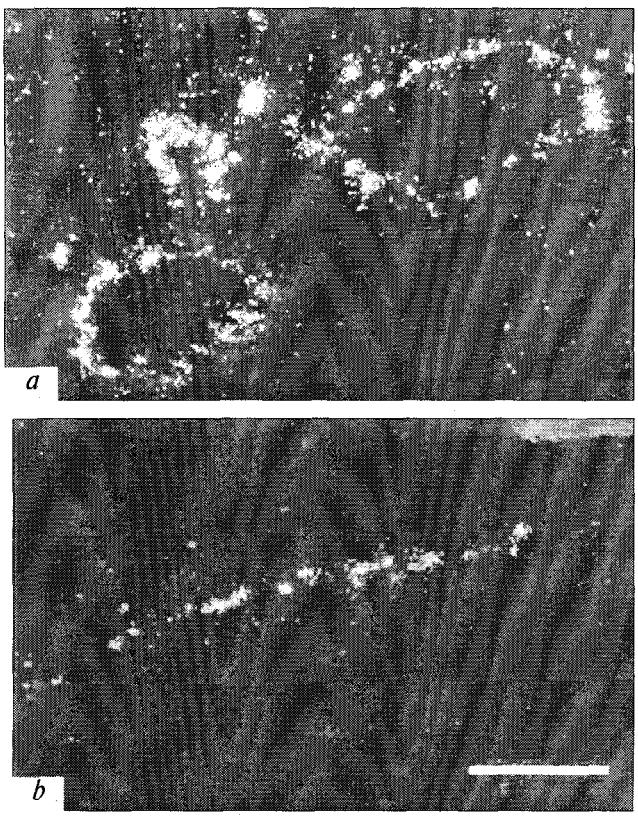

Fig. 2. Localization of autoradiographic label corresponding to cyclooxygenase-2 mRNA in meningeal (*a*) and parenchymal (*b*) cerebral vessels in rats injected with high dose of lipopolysaccharide. The mark corresponds to 250 μ.

duced by endothelial COG-2 contribute to manifestation of anorexia caused by high doses of LPS.

Our findings indicate that the vagus nerve activates central structures responsible for manifestation of anorexia during moderate abdominal inflammation. Severe inflammation accompanied by the appearance of pathogenic materials in the circulation activates a vagus-independent mechanism of prostaglandin secretion in the endothelium of cerebral vessels. Thus, immune stimulation of various CNS structures is mediated by several mechanisms depending on the intensity of inflammatory process.

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